Production Information

GeneDia™ 2x PCR Master Mix

1.5 mM $MgCl_2$final concentration

Storage Temperature -20 C

GeneDia 2x PCR Master Mix	1.5 mM $MgCl_2$
colour	RED
Lot No.	MM01100
Content	1 ml

Product Description

GeneDia™ 2x PCR Master Mix RED is a ready-to-use 2x reaction mix with the Taq DNA polymerase, the NH4+ buffer system, dNTPs and magnesium chloride present. Each reaction requires 10 µl of the 2x Master Mix RED. Simply add primers, template and water to a total reaction volume of 20 µl to successfully carry out primer extensions and other molecular biology applications.

GeneDia™2x PCR Master Mix RED offers several advantages. Set up time is significantly reduced. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time. There is no need to buy and use separate loading dyes. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The red dye front runs at 1000 – 2000 bp on a 0.5 – 1.5% agarose gel.

Precautions and Disclaimer

For Research Use Only.

Composition of the 2x PCR Master Mix RED

(1.5 mM $MgCl_2$ final concentration)

- Tris-HCl pH 8.5, (NH4)2S04, 3 mM MgCl2, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Taq DNA polymerase
- Inert red dye
- Stabilizer

Quality Control

Genedia™ 2x PCR Master Mix RED is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Storage/Stability

Genedia™ 2x PCR Master Mix RED should be stored at -20˚C. Thawed material kept on ice can be aliquoted and re-frozen up to two times.

Pre-procedure Considerations

Primers and Probes

The design of primers and probes is critical especially for successful multiplex PCR.

Design primers with similar annealing temperature.

Analyse primer and probe sequences to avoid primer/probe hairpins, homo- or heterodimers, or any primer/probe complementarity across the targets.

Optimization of primer and probe concentrations is highly recommended.

Preventing Template Cross-Contamination

Due to the high sensitivity of quantitative PCR there is a risk of contaminating the reactions with the products of previous runs.

Procedure:

This protocol serves as a guideline to ensure optimal PCR results when using 2x PCR Master Mix RED. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw 2x PCR Master Mix RED and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice.
2. Prepare a reaction mix. Table 1 shows the reaction set up for a final volume of 20 µL.

Component	Vol./reaction*	Final concentration*
GeneDia™ 2x PCR Master Mix	10 µl	**1x**
Forward Primer	0.5 µl	0.5 µl of 10 µM/µl final concentration (0.2 µM/µl)
Reverse Primer	0.5 µl	0.5 µl of 10 µM/µl final concentration (0.2 µM/µl)
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	20 µl	-

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the reaction mix up and down a few times.
4. Add template DNA to the individual tubes containing the reaction mix.
5. Program the thermal cycler according to the manufacturer´s instructions. See table 2 for an example. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Cycles	Duration of cycle	Temperature
1	**15 minutes**	**95 ° C**
30	**30-50 seconds** **30 seconds** **30 seconds**	**95 ° C** **60-55 ° C** **72 ° C**

6. Place the tubes in the thermal cycler and start the reaction.
7. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

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